A Phylogenetic Analysis of Nesting Behavior in the Genus *Osmia* (Hymenoptera: Megachilidae)

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ABSTRACT   Cladistic analysis is used to study the evolution of 29 biological characters related to nesting behavior, nest provisioning, oviposition, cocoon spinning, defecation, and life cycle (nest characters) in 11 species of *Osmia* bees (Hymenoptera: Megachilidae) and two outgroup genera. A molecular phylogeny based on 38 allozyme loci-as-characters and a combined character phylogeny are produced to compare with the nest character phylogeny. All phylogenetic trees support the monophyly of the genus *Osmia* and the subgenus *Osmia (Osmia)* with *Osmia ribifloris* Cockerell basal to the other species in this subgenus. The subgenus *Helicosmia* (=*Chalcosmia*) is resolved as a sister clade to *O. (Osmia)* in the molecular tree and as sister to the subgenus *Cephalosmia* in the nest character and combined trees. Incomplete biological information from additional species of all three subgenera is provided to support the consistency of nest characters at the subgeneric level and their use in the establishment of subgeneric phylogenies in the family Megachilidae.

KEY WORDS   bees, *Osmia*, nesting phylogeny, molecular characters, behavioral characters

THE IDEA THAT behavior evolves in essentially the same fashion as morphology justifies the use of behavioral characters both on their own and combined with morphological or molecular characters in the inference of phylogenies (Wenzel 1992, de Queiroz and Wimberger 1993, Proctor 1996). This possibility has long been recognized by ethologists (Whitman 1899, Heinroth 1911, Tinbergen 1959), but phylogenetic studies using behavioral characters are still scarce (Wenzel 1992, de Queiroz and Wimberger 1993, Proctor 1996). It has been argued that behavioral characters are difficult to homologize and are more evolutionarily labile (subject to convergence and reversal) than morphological characters (Atz 1970, Baroni Urbani 1989), but some studies have shown similar levels of homoplasy between behavioral and morphological or molecular data sets (McLennan et al. 1988, Arntzen and Sparreboom 1989, Coddington 1990, Prum 1990, de Queiroz and Wimberger 1993, Proctor 1996). The limited use of behavioral characters in phylogenetic studies may be attributed to the difficulty of collecting behavioral data, a task that often requires large amounts of observation time. Sometimes, behavioral data can be obtained through the study of structures that are the result of particular behaviors. In nesting Aculeate Hymenoptera (ants, bees, wasps), a considerable amount of behavioral (nest building, provisioning, oviposition, cocoon spinning) as well as physiological (life cycle, defecation) information can be obtained through nest analysis. Thus, behavioral characters, often related to nesting activities and nest architecture,

have been used in aculeate Hymenoptera phylogenetic studies, mostly in combination with larger numbers of morphological characters (Carpenter 1982, 1987, 1988; Carpenter and Cumming 1985; McGinley and Rozen 1987; Baroni Urbani 1989, 1993; Alexander 1990, 1991; Rozen 1991, Wenzel 1993). Other Aculeate studies have traced the evolution of behavioral traits on phylogenies obtained from morphological or molecular characters (Packer 1991, Carpenter et al. 1993, Chavarria and Carpenter 1994, Engel and Schultz 1997).

Bee (Apoidea) nesting behavior may be classified as burrowing (carpenters or miners) and nonburrowing, according to whether species do or do not excavate their own nests (Malyshev 1935, Stephen et al. 1969, O'Toole and Raw 1991). Among the latter, some species use preestablished cavities, and others build their cells in more or less exposed situations. The primitive condition in the family Megachilidae is to nest in burrows, excavated in either soil (Fideliinae - Rozen 1970, 1973, 1977; McGinley and Rozen 1987; - *Trachusa* Malyshev 1935, Michener 1941, Westrich 1989) or wood (Lithurgini - Malyshev 1935, Cros 1939, Brach 1978). However, three or more types of nests (including nests in wood cavities, in snail shells, in cracks in rocks, burrows in the ground, burrows in stems, and exposed nests) are found in several derived genera (*Anthidium, Osmia, Hoplitis, Megachile* subgenera *Litomegachile* and *Delomegachile*) (Malyshev 1935, Stephen et al. 1969, Eickwort et al. 1981, Westrich 1989). The absence of secreted substances in nest construction and the frequent incorporation of external materials, in cavity-nesters as well as burrowing species, is characteristic of the Megachilidae (Stephen et al. 1969). The use of certain nesting materials has appeared several times independently throughout the

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evolution of the family. Soil use is found in species of *Osmia, Hoplitis, Chelostoma, Megachile* subgenera *Eumegachile* and *Chalicodoma*, resin in species of *Heriades, Chalicodoma, Anthidium, Trachusa,* and *Hoplitis*, and masticated leaf in species of *Osmia, Ashmeadiella, Hoplitis,* and *Anthidium* (Malyshev 1935, Krombein 1967, Stephen et al. 1969, Rust 1980, Westrich 1989, Bosch et al. 1993). Pollen specialization is another plastic character. Most Megachilidae are polylectic, but oligolecty appears to be frequent in the primitive Fideliinae and Lithurgini (Rozen 1970, 1977; Brach 1978; Roberts 1978; Parker and Potter 1973; Yáñez 1997) and is common in *Trachusa, Anthidium, Chelostoma, Heriades, Osmia,* and *Hoplitis* (Rust 1974, Westrich 1989, Cane 1996, Müller 1996). Despite this apparently high degree of homoplasy, Torchio (1989) showed that biological characters could be used to characterize *Osmia* subgenera.

In this work, we used a cladistic analysis to study the evolution of 29 characters related to nest construction, nest provisioning, oviposition, life cycle, defecation, and cocoon spinning (nest characters) in 11 species of *Osmia*, representing three different subgenera. Two outgroup species, *Hoplitis adunca* (Panzer) and *Megachile* (*Chalicodoma*) *angelarum* (Cockerell), are included in the analyses. We provide a molecular phylogeny based on 38 allozyme loci-as-characters that we compare with the behavioral phylogeny and the combined phylogeny of the 13 species. The two objectives of this article are as follows: (1) discuss the usefulness of nest characters in the establishment of phylogenetic hypotheses in the Megachilidae and (2) discuss the evolution of nesting behavior and related biological features among the three *Osmia* subgenera considered and in relation to the rest of the Megachilidae.

Materials and Methods

Species Studied. The genus *Osmia* contains ≈500 species found in the Holarctic (Rust 1974). All *Osmia* are solitary, and many nest in preestablished cavities. Nest-traps (Krombein 1967) consisting of cavities of different diameters drilled in wooden blocks were placed in different locations in the United States, Japan, France, and Spain (Table 1). Bee nests obtained in the cavities were taken to the laboratory, where they were dissected. We collected data on nest architecture, nesting materials, provision structure and oviposition, cocoon structure, shape and position of fecal particles, and progeny developmental stages. Progeny were reared to the adult stage and frozen for molecular analyses. We obtained nests and adult bees for 11 species of *Osmia* in three different subgenera [*Osmia, Helicosmia* (=*Chalcosmia*), and *Cephalosmia*] (Table 1). The subgenus *Chalcosmia* has been recently homologized with the subgenus *Helicosmia* (Griswold and Michener 1997, Michener 2000). *Megachile angelarum* and *Hoplitis adunca* were used as outgroup species. Outgroup status was established according to Roig-Alsina and Michener (1993). Based on adult morphology, the Megachilidae are considered a

Table 1. Bee species, locations and number of specimens used in the molecular analysis

Species	Location	No. of specimens (females, males)
Osmia (Osmia)		
cornifrons (Radoszkowski)	Matsue, Japan	10, 10
cornuta Latreille	Girona, Spain	5, 5
	Avignon, France	15, 5
lignaria Say	Reno, Nevada, USA	45, 45
ribifloris Cockerell	Reno, Nevada, USA	10, 15
rufa (L.)	Girona, Spain	27, 15
	Avignon, France	21, 15
taurus Smith	Matsue, Japan	10, 10
tricornis Latreille	Girona, Spain	15, 10
Osmia (Helicosmia)		
fulviventris Panzer	Girona, Spain	10, 10
	Avignon, France	5, 4
latreillei Spinola	Girona, Spain	10, 10
	Avignon, France	-, 5
Osmia (Cephalosmia)		
californica Cresson	Logan, Utah, USA	15, 15
montana Cresson	Logan, Utah, USA	15, 10
Megachile		
angelarum (Cockerell)	Lake City, California, USA	15, 5
Hoplitis		
adunca (Panzer)	Valladolid, Spain	10, 5

monophyletic group divided into two subfamilies, the Fideliinae and the Megachilinae, with the latter containing four tribes, the Lithurgini (basal), Anthidiini, Megachilini, and Osmiini. Although the status of the last three tribes remains unresolved when larval characters are used, adult characters place *Osmia* and *Hoplitis* within Osmiini, and Megachilini as the sister tribe to Osmiini (Roig-Alsina and Michener 1993). Some authors consider *Hoplitis* as a subgenus within *Osmia* (Westrich 1989).

Molecular Characters. Adult females were homogenized in 0.03 ml of cold extraction buffer (Tris HCl 0.05 M, pH 7.0; May 1992). After 20 min of cold incubation and low-speed centrifugation, the supernatant was pipetted into 1.5-ml Eppendorf tubes and stored at −80°C until used for electrophoresis, which occurred within 3–4 wk after preparation. Supernatant was applied to 14% horizontal starch gels (50% Counaught and 50% Sigma, St. Louis, MO) using filter-paper wicks (Whatman #3, Whatman, Hillsboro, OR), and gels ran for ≈5 h. We used the methods and staining procedures described by May (1992). *Osmia lignaria* allele frequencies (R.R., unpublished data) and individuals were used as a standard (five individuals per gel) for loci and alleles determination in all gels. Thirty-eight loci in 26 enzymes system (Table 2) were scored as loci-as-characters using the approach of Mardulyn and Pasteels (1994). The three rules developed by Mardulyn and Pasteels (1994) were used to reconstruct the most-parsimonious trees for each locus. Allele gains and losses were treated equal in the reconstruction of steps from ancestral nodes to the

Table 2. Loci and electrophoretic conditions used to assay bee species

Locus	Enzyme	EC No.	Buffer[a]
AK-1,2	Adenylate kinase	2.7.4.3	C
CK-1,2	Creatine kinase	2.7.3.2	TC-1
DIA-1	Diaphorase (NADH)	1.8.1.4	R
EST-1	Esterase	—	Tris/HCl
ESTF-1,2	Fluorescent esterase	—	Tris/HCl
FBP-1	Fructose-bisphosphatase	3.1.3.11	R
GAM.-1	Galactosaminidase	—	4
GAPDH-1	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	4
GDA-1	Guanine deaminase	3.5.4.3	4
GK-1	Glucokinase	2.7.1.2	R
GP-1,2	General protein	—	R
GPI-1,2	Glucosephosphate isomerase	5.3.1.9	C
GR-1	Glutathione reductase	1.6.4.2	R
G3P-1	Glycerol-3-phosphate dehydrogenase	1.1.1.8	4
G6PDH-1	Glucose-6-phosphate dehydrogenase	1.1.1.49	TC-1
HA-1	Hexosaminidase	3.2.1.52	R
HBDH-1	Hydroxybutyric dehydrogenase	1.1.1.30	TC-1
IDH-1	Isocitrate dehydrogenase	1.1.1.42	4
MDH-1	Malate dehydrogenase	1.1.1.37	C
ME-1	Malic enzyme	1.1.1.40	R
MPI-1	Mannosephosphate isomerase	5.3.1.8	C
ODH-1	Octanol dehydrogenase	1.1.1.73	C
PEP-1,2,3,4,5	Peptidase	3.4.–.–	R
PGD-1	Phosphogluconate dehydrogenase	1.1.1.43	C
PGM-1,2,3	Phosphoglucomutase	5.4.2.2	4
SOD-1,2	Superoxide dismutase	1.15.1.1	Tris/HCl

[a] From May 1992.

taxa to produce the shortest possible tree for each locus.

Nest Characters. All species studied are cavity nesters, and five of the characters identified (characters 8–12, Table 3) may not be easily applicable to other nesting types. Cavity-nesting Megachilidae tend to build linear series of cells separated by cell partitions with a closing plug at the cavity entrance. Each cell is provisioned with a mass of pollen and nectar and, in most cases; one egg only per cell is deposited. Our nest and cell terminology followed Krombein (1967). Our observations were supplemented with published data (Rust 1974, 1986; Maeta 1978; Torchio 1989; Bosch et al. 1993; Vicens et al. 1993) to identify 29 nest characters for each species. Because character states did not always coincide in the two outgroup species, character polarity was based on the Lithurgini. The Lithurgini are unequivocally ancestral within the Megachilinae (Peters 1972, Michener 1983, Roig-Alsina and Michener 1993), and represent a biologically consistent group (Malyshev 1935; Cros 1939; Houston 1971; Rozen 1973; Brach 1978; Parker and Potter 1973; Roberts 1978; Garófalo et al. 1981, 1992; Camillo et al. 1983, 1994; Yàñez 1997). To assess the subgeneric consistency of the nest characters, information was gathered for 17 additional species, three *Cephalosmia*, 10 *Helicosmia*, and four *O.* (*Osmia*) (Malyshev 1935; Hartman 1944; Grandi 1964; Taséi 1972, 1976; Hawkins 1975; Raw 1974; Rust 1974; Maeta

1978; Parker 1980, 1985; Westrich 1989; Vicens et al. 1993; and unpublished data). These species were not used in the cladistic analyses because none of the molecular characters and only some of the nest characters could be scored, but they provided supporting evidence to our results.

Combined Characters. The informative loci-as-characters and nest characters were used together in a combined analysis.

Parsimony Analysis. Character sets were unweighted and unordered. Molecular, nest character, and combined data sets were analyzed with PAUP 3.01 using heuristic search and TBR branch swapping (Swofford 1993). Bootstrap analyses using 100 iterations were used to obtain confidence limits on individual clades. Trees were rooted according to Roig-Alsina and Michener's (1993) phylogeny.

Results

Molecular Character Phylogeny. Parsimony analysis of 31 informative loci (*Appendix 1*) produced six trees (length = 123; consistency index [CI] = 0.699; retention index [RI] = 0.619). The strict consensus tree supports the monophyly of *Osmia* and the subgenera *Cephalosmia, Helicosmia,* and *Osmia,* with the two latter as sister clades and *Osmia ribifloris* as the basal species in the *O.* (*Osmia*) clade (Fig. 1). The bootstrap trees (mean length, 129; range, 77–175; CI = 0.597; RI = 0.469) supported the genus *Osmia* (82%) and the subgenera *Osmia* (65%), *Cephalosmia* (99%), and *Helicosmia* (70%). The genus *Osmia* was supported by changes at five loci (EST1, ESTF1, GPI1, ME1, PEP5). The subgenus *Osmia* was supported by loci GK1, MPI1, PEP2, SOD2, the subgenus *Cephalosmia* by loci DIA1, FBP1, GK1, G6PDH1, HBDH1, PEP2, and the subgenus *Helicosmia* by loci GP1, PEP4.

Nest Character Phylogeny. Parsimony analysis of 21 informative nest characters (*Appendix 2 and 3*) produced three trees (length, 41; CI = 0.805; RI = 0.873). The strict consensus tree resolved the genus *Osmia* and the subgenus *Osmia. Osmia ribifloris* (Cockerell) is again basal to the other *O.* (*Osmia*) species, of which only *O. rufa* (L.) and *O. taurus* Smith are resolved as sister species. The two *Cephalosmia* species form a trichotomy with the two *Helicosmia*, which are grouped together (Fig. 2). Bootstrap trees (mean length, 43; range, 34–92; CI = 0.837; RI = 0.887) support the genus *Osmia* (71%), the subgenus *Osmia* (70%), the pairing of the two *Helicosmia* (91%) and the *Helicosmia–Cephalosmia* clade (94%). Bootstrap trees also support the basal position of *O. ribifloris* (80%) in the *O.* (*Osmia*) clade and the *O. rufa–O. taurus* pairing (84%) (Fig. 2). The genus *Osmia* was supported by wintering in the adult stage (3-1), the cell walls not lined (9-0), cocoon nipple present and raised (26-2), and the presence of a thick middle layer in the cocoon (28-1). The subgenus *Osmia* was supported by adult activity in the spring (1-1), the cocoon attached only to the posterior cell partition (25-0), and cocoon with a silky outer layer (29-1). The *Cephalosmia-Helicosmia* clade was supported by oligolecty (13-

Table 3. Nesting character state frequency in *Osmia* (*Osmia*) (*n* = 11 species), *Osmia* (*Helicosmia*) (*n* = 12 species), and *Osmia* (*Cephalosmia*) (*n* = 5 species)

Character	O. (Osmia): 11 spp.						O. (Helicosmia): 12 spp.						O. (Cephalosmia): 5 spp.						
	0	0–1	1	2	3	?	0	0–1	1	2	3	?	0	0–1	1	2	2–3	3	?
Activity (1)			11				7	2	3				5						
Voltinism (2)	11						3		5	1		3			3				2
Wintering (3)			10		1			5	3			4		3	1				1
Proterandy (4)	10				1		9		1			2	3						2
Nest Site (5)			10	1					11	1			4					1	
Nest materials (6)	10				1		9					3	3		2				
Main material (7)			8	2		1				11		1					2	3	
Partitions (8)	9				1		9					3			4				
Cell walls (9)	9				1		9					3		4					
Plug materials (10)	9				1		9					3		4					
Plug position (11)	8		1		1		1		7			4			3				1
Vestibule (12)		9			1		1		8			3			3				1
Foraging (13)	1		10				8		3			1	5						
Provision texture (14)	2		7		2				4	4		4					2		3
Provision shape (15)			10		1		4		3			5	4		1				
Egg placement (16)				10		1			4	4		4	5						
Egg laying (17)	10				1		8					4	3						2
Number eggs (18)	10				1		9					3	5						
Fecal placement (19)	9					2	9					3	5						
Fecal consistency (20)	9					2			9			3			4				1
Fecal cross-section (21)	6	1				4	1	2	6			3			5				
Fecal shape (22)		1	6			4	3	4	2			3	1	1					3
Fecal surface (23)		1	6			4	6	2	1			3	5						
Fecal color (24)	1		6			4	9					3	5						
Cocoon attach (25)			9	2			9					3	4						1
Cocoon nipple (26)			10		1				1	8		3				5			
Cocoon cap (27)	9		1		1				9			3			5				
Cocoon middle (28)			8		3				9			3			5				
Cocoon outer (29)			10		1				9			3	5						

Numbers indicate the number of species within a subgenus sharing a particular state. *O.* (*Osmia*) species: *O. cerinthidis* Morawitz, *O. cornifrons*, *O. cornuta*, *O. excavata* Alfken, *O. lignaria*, *O. mustelina* (=*emarginata*), *O. pedicornis* Cockerell, *O. ribifloris*, *O. rufa*, *O. taurus*, *O. tricornis*. *O.* (*Helicosmia*) species: *O. caerulescens* L., *O. chalybea* Smith, *O. coloradensis* Cresson, *O. dimidiata* Morawitz, *O. jacoti* (=*imaii* Hirashima), *O. fulviventris*, *O. georgica* Cresson, *O. latreillei*, *O. leaiana* Kirby, *O. melanogaster* Spinola, *O. orientalis*, *O. texana* Cresson. *O.* (*Cephalosmia*) species: *O. californica*, *O. grinnelli* Cresson, *O. marginipennis* Cresson, *O. montana*, *O. subaustralis* Cockerell. Characters 8–12 were not scored for non-cavity-nesters, *O.* (*Osmia*) *cerinthidis* and *O.* (*Cephalosmia*) *marginipennis*.

0), the pollen provisions reworked after the last pollen load, with a wet core and a dry outer layer (14-3), and the provisions filling the whole cell (15-0), the fecal particles smeared (20-1) and cocoon nipple hidden by a silky cap (27-1). The only character unique to the two *Cephalosmia* species is oviposition in an egg cavity (16-0). *Helicosmia* is supported by the fecal pellets with truncated tips (22-0). Supporting evidence from other species in the three subgenera is provided in Table 3. For many of the characters, where information is known, character states are relatively consistent within each subgenus but differ between subgenera.

Combined Data Phylogeny. Parsimony analysis of the combined data sets produced six trees (length, 156; CI = 0.692; RI = 0.692). The strict consensus tree supports the genus *Osmia* and the three subgenera (Fig. 3). Within the *O.* (*Osmia*) clade *Osmia ribifloris* is basal to *O. cornifrons* (Radoszkowski) and to the other unresolved *O.* (*Osmia*) species. The bootstrap trees (mean length, 167; CI = 0.665; RI = 0.650) support the genus *Osmia* (67%) and the subgenera *Osmia* (95%), *Helicosmia* (84%), and *Cephalosmia* (100%). Bootstrap trees also support the clade *Heli-cosmia*–*Cephalosmia* (57%) and the basal position of *O. ribifloris* (87%) and *O. cornifrons* (51%) within *O.*

(*Osmia*) (Fig. 3). Both molecular and nest character state changes that support the various species and clades are shown in Fig. 3.

Discussion

Both our molecular and nest phylogenetic trees support the monophyly of the genus *Osmia* and the subgenus *O.* (*Osmia*) and place the two *Helicosmia* as sister species. They also coincide in placing *O. ribifloris* basal to the other *O.* (*Osmia*). The main difference between the two trees is the placement of *Helicosmia*, sister to *O.* (*Osmia*) in the molecular tree and sister to *Cephalosmia* in the nest character tree. *Helicosmia* and *Cephalosmia* are indeed very similar biologically and, as a result, biological features outweigh molecular characters in the combined analysis, where these two subgenera are resolved as sister clades. The only other cladistic analysis of *Osmia* is found in Peters (1978). He used morphological characters to analyze 16 western Palearctic species in the subgenera *Orientosmia*, *Monosmia*, and *Osmia* (including *O. rufa*, *O. cornuta* Latreille, and *O. tricornis* Latreille from this study). His analysis resolved the three subgenera, and placed the three mentioned species in one of two
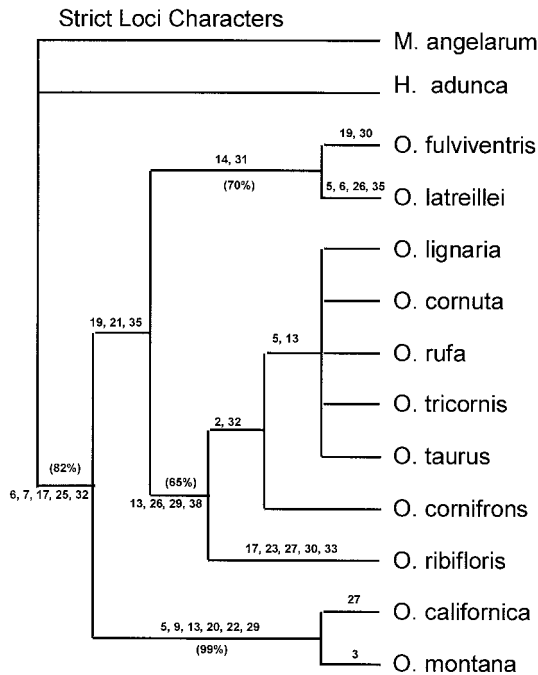
Strict Loci Characters

Fig. 1. Strict consensus of the six most parsimonious trees of 123 steps derived from heuristic analysis (unweighted, unordered) of 31 informative loci-as-characters. *Megachile angelarum* and *Hoplitis adunca* were designated outgroup members. Numbers above branch points are supporting characters, and numbers in parentheses are percentage values for clades found in bootstrap majority rule trees.

Strict Nest Characters

Fig. 2. Strict consensus tree of the three most parsimonious trees of 41 steps derived from heuristic analysis (unweighted, unordered) of 21 informative nest characters. *Megachile angelarum* and *Hoplitis adunca* were designated outgroup members. Numbers above branch points are supporting characters, and numbers in parentheses are percentage values for clades found in bootstrap majority rule trees.

polytomous clades within the subgenus *O.* (*Osmia*). Unfortunately, he did not provide characters or a character matrix that could be combined with our data for these three species.

The genus *Osmia* shows several derived morphological traits within the Megachilidae (Roig-Alsina and Michener 1993) and with *Hoplitis* was placed in the tribe Osmiini (Michener 1941a, Sinha 1958, Roig-Alsina and Michener 1993). The nest characters used in our study also suggest a derived phylogenetic status for *Osmia.* Overwintering as larvae (prepupae) is the primitive condition in the Megachilidae (Fideliinae - Rozen 1970, McGinley and Rozen 1987; Lithurgini - Cros 1939, Parker and Potter 1973, Roberts 1978, Camillo et al. 1983, Garófalo et al. 1981, 1992). The larval stage is the most common overwintering state in other megachilid genera *(Trachusa, Anthidium, Megachile)* (Michener 1941b, MacSwain 1946, Krombein 1967, Parker 1987, Westrich 1989, Bosch et al. 1993), and other Osmiini genera *(Hoplitis, Heriades, Proteriades, Anthocopa, Chelostoma)* (Krombein 1967; Clement and Rust 1975, 1976; Rust 1980; Parker 1977, 1978, 1988; Westrich 1989). Conversely, most *Osmia* overwinter as adults, or facultatively as adults and prepupae (Fye 1965; Medler 1967; Krombein 1967; Rust and Clement 1972; Rust et al. 1974; Frohlich 1983; Cripps and Rust 1985; Parker 1984, 1986; Torchio 1989; Westrich 1989; Vicens et al. 1993; this study). Probably in relation to
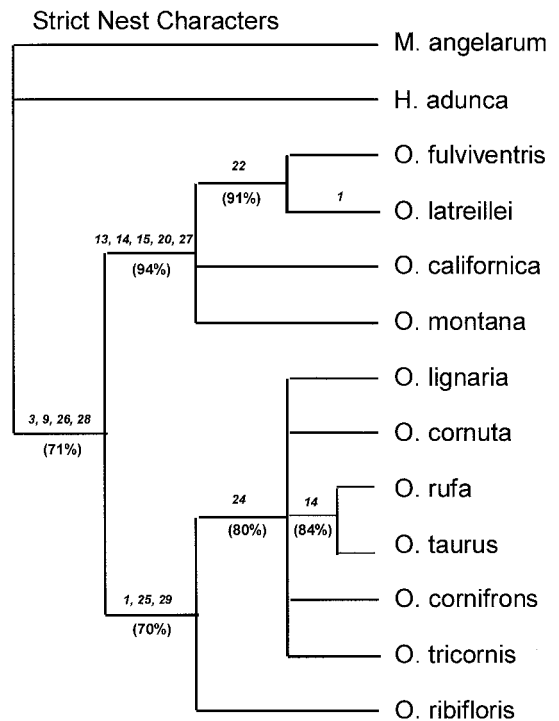
their wintering in the adult stage, most *Osmia* species fly early in the year compared with other Megachilidae. Up to 85% of 20 *Osmia* species but only 15.3% of 72 non-*Osmia* megachilids from southeastern Germany start flying in May or earlier (Westrich 1989).

The genus *Osmia* is also characterized by building unlined cells, delimited by simple cell partitions composed of only one material in most cases. The absence of cell lining to isolate immature stages from the nesting substrate is compensated for with the spinning of a thick, multilayered cocoon with a thick brownish layer made of salivary matrix (Torchio 1989) and a strong apical nipple. This cocoon structure contrasts with that of many megachilid genera, including the ancestral Lithurgini (Brach 1978, Parker and Potter 1973, Roberts 1978) and many other Osmiini *(Ashmeadiella, Heriades, Hoplitis, Chelostoma)* (Krombein 1967; Clement and Rust 1975, 1976; Rust 1980; Westrich 1989; Parker 1988; Bosch et al. 1993), which spin thin, translucent cocoons with absent or weak nipples.

Within the genus *Osmia*, nest characters indicate a more derived status for *O.* (*Osmia*) than for *Cephalosmia* or *Helicosmia*. The latter two subgenera are intermediate between *O.* (*Osmia*) and other Megachilidae in wintering stage and activity period. All *O.*
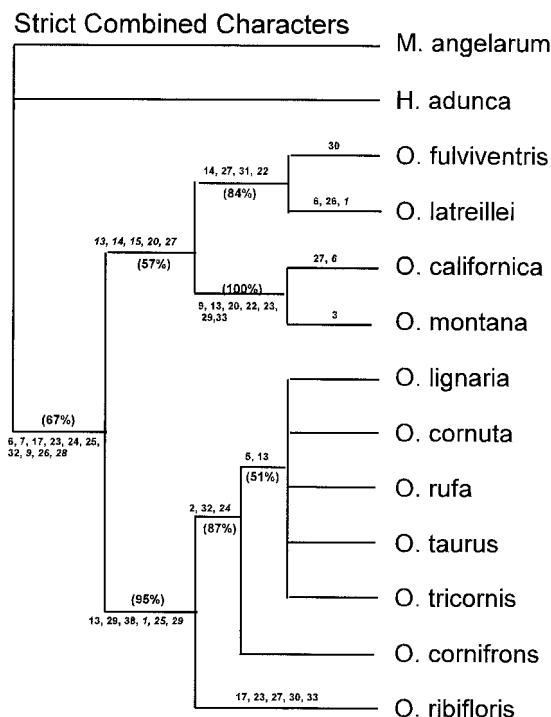
Strict Combined Characters

Fig. 3. Strict consensus of the six most parsimonious trees of 156 steps derived from heuristic analysis (unweighted, unordered) of the combined 31 informative loci-as-characters and 21 informative nest characters. *Megachile angelarum* and *Hoplitis adunca* were designated outgroup members. Numbers above branch points are supporting characters (nest characters in italics), and numbers in parentheses are percentage values for clades found in bootstrap majority rule trees.

(*Osmia*) overwinter strictly as adults, whereas most *Cephalosmia* and *Helicosmia* species overwinter facultatively as prepupae and adults (Table 3). *O. (Osmia)* species tend to fly earlier than *Cephalosmia* or *Helicosmia* (Table 3), and this tendency is apparent within geographical regions as diverse as the Great Basin area of the United States, Japan, southeastern Germany, and northeastern Spain (Rust 1974, Maeta 1978, Westrich 1989, Vicens et al. 1993; and unpublished data). Cocoon structure is also more derived in *O. (Osmia)* than in *Cephalosmia* and *Helicosmia.* Cocoons of these two subgenera are composed of an outer thin and fragile translucent layer, a thick middle and consistent brownish layer, and an inner thin layer composed of densely woven silk strands. This last layer is often incomplete, not reaching the basal tip of the cocoon. In *O. (Osmia)* cocoons have an additional outer layer of loose silk strand meshwork, and the inner layer is, in most cases, complete. Like in other cavity-nesting Osmiini (*Hoplitis, Ashmeadiella, Heriades,* and *Chelostoma*) (Krombein 1967, Clement and Rust 1976, Westrich 1989, Bosch et al. 1993) and Megachilidae (*Lithurgus, Megachile,* subgenera *Eumegachile, Chalicodoma*) (Cros 1939; Michener 1953; Krombein 1967; Houston 1971; Brach 1978; Garófalo et

al. 1981, 1992; Frohlich and Parker 1983; Kim 1992; Bosch et al. 1993; this study) the cocoon is in contact with both the posterior and anterior partitions of the cell in *Cephalosmia* and *Helicosmia,* whereas it is only attached to the posterior partition in *O. (Osmia).*

Cephalosmia and *Helicosmia* show striking biological similarities in their life histories, nesting behavior, pollen specialization, and cocoon and fecal pellet structure. *Helicosmia,* however, is more polymorphic (Table 3) and includes some species, primarily *O. caerulescens* (L.), *O. orientalis* Benoist, and *O. jacoti* Cockerell, that are similar to *O. (Osmia),* or intermediate between *O. (Osmia)* and *Cephalosmia,* in activity period, voltinism, wintering stage, pollen specialization, provision structure, egg placement, and fecal pellet structure. *Osmia caerulescens, O. orientalis,* and *O. jacoti* are morphologically distinct from other *Helicosmia* (Rust 1974, Tkalcu 1975). They lack the development of the apical margin of the clypeus and the projections at the base of the mandibles typical of other female *Helicosmia* (Yasumatsu and Hirashima 1950, Rust 1974, Tkalcu 1975). *Osmia orientalis* females lack a diagnostic subgeneric character and are only placed in *Helicosmia* based on the male's subgeneric characters (Yasumatsu and Hirashima 1950). Nest character 16 (egg placement) could be ordered assuming that egg laying in a depression on the surface of the provision (some *Helicosmia*) is an intermediate state between egg laying in a chamber (*Cephalosmia*) and egg laying on the surface of the provision [*O. (Osmia)* and some *Helicosmia*]. However, our two phylogenetic hypotheses [*Helicosmia* sister to *O. (Osmia)* and *Helicosmia* sister to *Cephalosmia*] would be equally parsimonious to this change.

Both the molecular and the nest character analyses support the basal position of *O. ribifloris* within the *O. (Osmia)* clade, indicating a transition from leaf to mud use in *O. (Osmia).* Biologically, *O. ribifloris* is unmistakably similar to the other *O. (Osmia)* in all characters except in use of leaf material, nest plug position and fecal particle color. These three characters are shared with *Cephalosmia* and *Helicosmia. Osmia ribifloris* is also the only *O. (Osmia)* in which the inner layer of the cocoon was incomplete in most of the specimens we examined as in all *Cephalosmia* and *Helicosmia.* Morphologically, female *O. ribifloris* differ from other female *O. (Osmia)* species in lacking the tubercle modifications of the clypeus. However, both males and females show all other unique subgeneric characters (Rust 1974). Torchio (1989) provided detailed behavioral descriptions of the use of clypeal modifications to smooth the surface of cell partitions in the mud user *O. lignaria.* The only other species in the subgenus using leaf material for cell construction is *O. mustelina* Gerstaecker (=*emarginata* Lepeletier) (Grandi 1964), which also lacks clypeal tubercles and is morphologically very similar to *O. ribifloris* (Rust 1974). Masticated leaf is the most commonly used nesting material among *Osmia* in other subgenera (Krombein 1967, Maeta 1978, Parker and Tepedino 1982, Frohlich 1983, Westrich 1989), including *Cephalosmia* and *Helicosmia* (Table 3) as well as several

subgenera considered morphologically primitive *(Diceratosmia, Nothosmia, Chenosmia, Euthosmia)* (Sinha 1958). It has been suggested that *O. ribifloris* could be placed (with *O. mustelina, O. nigrohirta* Friese, and other species) in the subgenus *Aceratosmia* based, in part, on the four-segmented rather than five-segmented maxillary palpi (Griswold and Michener 1997, Michener 2000). However, close examination of male and female *O. ribifloris, O. mustelina* and *O. nigrohirta* has revealed the presence of a fifth, although small, apical segment in the maxillary palpi.

Several studies have shown similar levels of homoplasy in behavioral and morphological character analyses (de Queiroz and Wimberger 1993, Proctor 1996). Many of the nest characters proposed in this study are relatively consistent at the generic or subgeneric levels (Table 3), indicating that they should prove useful in the establishment of generic and subgeneric phylogenies in the Megachilidae. At the specific level, however, several species have identical nest character scores, and as a consequence these characters were less useful to resolve the relative position of species within a subgenus. Thus, our behavioral tree only establishes the basal position of *O. ribifloris* and the pairing of *O. rufa* and *O. taurus* within the *O. (Osmia)* clade. More detailed behavioral observations might provide interspecific differences and resolve some of the polytomies obtained. For instance, although the cocoon nipple is structurally similar across several *O. (Osmia)*, the timing and the methods used by the larva for its construction differ among species (Torchio 1989). Similarly, the sequence of activities in the construction of an egg chamber is different between *O. montana* Cresson and *O. californica* Cresson (Torchio 1989).

The occurrence of species like *O. ribifloris, O. mustelina, O. caerulescens, O. orientalis,* and *O. jacoti,* which differ from other species in their respective subgenera for both morphological and biological characters, validates the use of biological/behavioral characters in the establishment of phylogenies. Future studies should include some of these species, as well as other *Osmia* subgenera, especially *Diceratosmia* and the North American endemic *Acanthosmioides* (Rust et al. 1974), both with distinctive subgeneric morphologies (White 1952, Sinha 1958).

Acknowledgments

References Cited

Alexander, B. A. 1990. A cladistic analysis of the nomadine bees (Hymenoptera: Apoidae). Syst. Entomol. 15: 121–152.

Alexander, B. A. 1991. A phylogenetic analysis of the genus *Apis* (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. 84: 137–149.

Arntzen, J. W., and M. Sparreboom. 1989. A phylogeny of the Old World newts, genus *Triturus:* biochemical and behavioral data. J. Zool. Lond. 219: 645–664.

Atz, J. W. 1970. The application of the idea of homology to behavior, pp. 53–74. *In* L. R. Aronson, E. Tobach, D. S. Lehrman, and J. S. Rosenblatt [eds.], Development and evolution of behavior: essays in honor of T. C. Schneirla. Freeman, San Francisco, CA.

Baroni Urbani, C. B. 1989. Phylogeny and behavioral evolution in ants, with a discussion of the role of behavior in evolutionary processes. Ethol. Ecol. Evol. 1: 137–168.

Baroni Urbani, C. B. 1993. The diversity and evolution of recruitment behavior in ants, with a discussion of the usefulness of parsimony criteria in the reconstruction of evolutionary histories. Insectes Soc. 40: 233–260.

Bosch, J., N. Vicens, and M. Blas. 1993. Analisis de los nidos de algunos Megachilidae nidificantes en cavidades preestablecides (Hymenoptera, Apoidea). Orsis 8: 53–63.

Brach, V. 1978. Notes on the biology of *Lithurgus gibbosus* Smith in Florida (Hymenoptera: Megachilidae). Bull. South. Calif. Acad. Sci. 77: 144–147.

Camillo, E., C. A. Garófalo, and J. C. Serrano. 1983. Preliminary notes on the biology of *Lithurgus huberi* (Hymenoptera: Megachilidae). Rev. Brazil. Biol. 43: 151–156.

Camillo, E., C. A. Garófalo, and J. C. Serrano. 1994. Nesting activities and nest reuse of *Lithurgus huberi* (Hymenoptera: Megachilidae). Rev. Brazil. Biol. 54: 183–194.

Cane, J. H. 1996. Nesting resins obtained from *Larrea* pollen host by an oligolectic bee, *Trachusa lareae* (Cockerell) (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 69: 99–102.

Carpenter, J. M. 1982. The phylogenetic relationships and natural classification of the Vespoidea (Hymenoptera). Syst. Entomol. 7: 11–38.

Carpenter, J. M. 1987. Phylogenetic relationships and classification of the Vespinae (Hymenoptera: Vespidae). Syst. Entomol. 12: 413–431.

Carpenter, J. M. 1988. The phylogenetic system of the Stenogastrinae (Hymenoptera: Vespidae). J. N.Y. Entomol. Soc. 96: 140–175.

Carpenter, J. M., and J. M. Cumming. 1985. A character analysis of the North American potter wasps (Hymenoptera: Vespidae; Eumeninae). J. Nat. Hist. 19: 877–916.

Carpenter, J. M., J. E. Strassmann, S. Turillazzi, C. R. Hughes, C. R. Solis, and R. Cervo. 1993. Phylogenetic relationships among paper wasp social parasites and their hosts (Hymenoptera: Vespidae; Polistinae). Cladistics 9: 129–146.

Chavarria, G., and J. M. Carpenter. 1994. Total evidence and the evolution of highly social bees. Cladistics 10: 229–258.

Clement, S. L., and R. W. Rust. 1975. The biology of *Hoplitis robusta* (Hymenoptera: Megachilidae). Entomol. News 86: 115–120.

Clement, S. L., and R. W. Rust. 1976. The nesting biology of three species of *Hoplitis* Klug. Pan-Pac. Entomol. 52: 110–119.

Coddington, J. A. 1990. Cladistics and spider classification: araneomorph phylogeny and the monophyly of orbweavers (Araneae: Araneomorphae: Orbiculariae). Acta Zool. Fenn. 190: 75–87.

Cripps, C., and R. W. Rust. 1985. Biology and subgeneric placement of *Osmia pikei* (Hymenoptera: Megachilidae). Entomol. News 96: 109–113.

Cros, A. 1939. Considerations generales sur le genre *Lithurgus* Latreille et biologie du *Lithurguss tabialis* Mor. (Hymenoptera: Apidae-Megachilinae). Bull. Soc. Fouad I Entomol. 22: 37–57.

Eickwort, G. C., R. W. Matthews, and J. M. Carpenter. 1981. Observations on the nesting behavior of *Megachile rubi* and *M. texana* with a discussion of the significance of soil nesting in the evolution of megachilid bees (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 54: 557–570.

Engel, M. S., and T. R. Schultz. 1997. Phylogeny and behavior in honey bees (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. 90: 43–53.

Frohlich, D. R. 1983. On the nesting biology of *Osmia (Chenosmia) bruneri* (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 56: 123–130.

Frohlich, D. R., and F. D. Parker. 1983. Nest building behavior and development of the sunflower leafcutter bee: *Eumegachile (Sayapis) pugnata* (Say) (Hymenoptera: Megachilidae). Psyche 90: 193–209.

Fye, R. E. 1965. Biology of Apoidea taken in trap nests in northwestern Ontario (Hymenoptera). Can. Entomol. 97: 863–877.

Garófalo, C. A., E. Camillo, M.J.O. Campos, R. Zucchi, and J. C. Serrano. 1981. Bionomical aspects of *Lithurgus corumbae* (Hymenoptera: Megachilidae), including evolutionary considerations on the nesting behavior of the genus. Rev. Brazil. Genet. 4: 165–182.

Garófalo, C. A., E. Camillo, M.J.O. Campos, and J. C. Serrano. 1992. Nest re-use and communal nesting in *Microthurge corumbae* (Hymenoptera: Megachilidae), with special reference to nest defense. Insectes Soc. 39:301–311.

Grandi, G. 1964. Studio sull'*Osmia emarginata* Lepel. Bull. Entomol. Bologna 27: 127–144.

Griswold, T. L., and C. D. Michener. 1997. The classification of the Osmiini of the eastern hemisphere (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 70: 207–253.

Hartman, C. G. 1944. Note on the habits of *Osmia georgica* Cresson as ascertained by the glass-tube method. Psyche 5: 162–165.

Hawkins, W. A. 1975. Nesting biology of *Osmia (Chalcosmia) georgica* (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 48: 493–499.

Heinroth, O. 1911. Beitrage zur Biologie, namentlich Ethologie und Psychologie der Anatiden. Verh. Ve. Int. Ornithol. Kongr. (Berl.) 1910: 589–702.

Houston, T. F. 1971. Notes on the biology of a lithurgine bee (Hymenoptera: Megachilidae) in Queensland. J. Aust. Entomol. Soc. 10: 31–36.

Kim, J. Y. 1992. Nest dimensions of two leaf-cutter bees (Hymenoptera: Megachilidae). Ann. Entomol. Soc. Am. 85: 85–90.

Krombein, K. V. 1967. Trap-nesting wasps and bees: Life histories, nests, and associates. Smithsonian Institution Press, Washington, DC.

MacSwain, J. W. 1946. The nesting habits of *Heteranthidium larreae* (Ckll.). Pan-Pac. Entomol. 22: 159–160.

Maeta, Y. 1978. Comparative studies on the biology of the bees of the genus *Osmia* of Japan, with special reference to their management for pollination of crops (Hymenoptera: Megachilidae) Kotoku Nat. Agric. Exp. Stn. 57: 1–221.

Malyshev, S. I. 1935. The nesting habits of solitary bees. Eos 2: 210–309.

Mardulyn, P., and J. M. Pasteels. 1994. Coding allozyme data using step matrices: defining new original states from the ancestral taxa. Syst. Biol. 43: 567–572.

May, B. 1992. Starch gel electrophoresis of allozymes, pp. 1-27. *In* A. R. Hoelzel [ed.], Molecular genetic analysis of populations: a practical approach. Oxford University Press, Oxford.

McGinley, R. J., and J. G. Rozen. 1987. Nesting biology, immature stages, and phylogenetic placement of the Palaearctic bee *Pararphophites* (Hymonoptera: Apoidea). Am. Mus. Nov. 2903: 1–21.

McLennan, M. J., D. R. Brooks, and J. D. McPhail. 1988. The benefits of communication between comparative ethology and phylogenetic systematics: a case study using gasterosteid fishes. Can. J. Zool. 66: 2177–2190.

Medler, J. T. 1967. Biology of *Osmia* in trap nests in Wisconsin (Hymenoptera: Megachilidae). Ann. Entomol. Soc. Am. 60: 338–344.

Michener, C. D. 1941a. A generic revision of the American Osmiinae with descriptive notes on Old World genera. Am. Midl. Nat. 26: 147–167.

Michener, C. D. 1941b. A synopsis of the genus *Trachusa* with notes on the nesting habits of *T. perdita* (Hymenoptera: Megachilidae). Pan-Pac. Entomol. 17: 119–125.

Michener, C. D. 1953. The biology of a leafcutter bee *(Megachile brevis)* and its associates. Univ. Kans. Sci. Bull. 35: 1659–1748.

Michener, C. D. 1983. The classification of the Lithurginae (Hymenoptera: Megachilidae). Pan-Pac. Entomol. 59: 176–187.

Michener, C. D. 2000. The bees of the world. John Hopkins Press, Baltimore, MD.

Müller, A. 1996. Host-plant specialization in Western Palearctic Anthidiine bees (Hymenoptera: Apoidae: Megachilidae). Ecol. Monogr. 66: 235–257.

O'Toole, C., and A. Raw. 1991. Bees of the world. Blandford Publishing, London.

Packer, L. 1991. The evolution of social behavior and nest architecture in sweat bees of the subgenus *Evylaeus* (Hymenoptera: Halictidae): a phylogenetic approach. Behav. Ecol. Sociobiol. 29: 153–160.

Parker, F. D. 1977. Nest of *Anthocopa enciliae* (Cockerell) and *A. elongata* (Michener) (Hymenoptera: Megachilidae). Pan-Pac. Entomol. 53: 47–52.

Parker, F. D. 1978. Biology of the bee genus *Proteriades* Titus (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 51: 145–173.

Parker, F. D. 1980. Nests of *Osmia marginipennis* Cresson with a description of the female (Hymenoptera: Megachilidae). Pan-Pac. Entomol. 56: 38–42.

Parker, F. D. 1984. The nesting biology of *Osmia (Trichinosmia) latisulcata* Michener. J. Kans. Entomol. Soc. 57: 530–436.

Parker, F. D. 1985. Nesting habits of *Osmia grinnelli* Cockerell (Hymenoptera: Megachilidae). Pan-Pac. Entomol. 61: 155–159.

Parker, F. D. 1986. Nesting associates, and mortality of *Osmia sanrafaelae* Parker. J. Kans. Entomol. Soc. 59: 367–377.

Parker, F. D. 1987. Nests of *Callanthidium* from block traps (Hymenoptera: Megachilidae). Pan-Pacific Entomol. 63: 125–129.

Parker, F. D. 1988. Nesting biology of two North American species of *Chelostoma* (Hymenoptera: Megachilidae). Pan-Pac. Entomol. 64: 1–7.

Parker, F. D., and H. W. Potter. 1973. Biological notes on *Lithurgus apicalis* Cresson. Pan-Pac. Entomol. 49: 294–299.

Parker, F. D., and V. J. Tepedino. 1982. Behavior of *Osmia (Nothosmia) marginata* Michener in the nest (Hymenoptera: Megachilidae). Pan-Pac. Entomol. 58: 231–235.

Peters, D. S. 1972. Über die Stellung von *Aspidosmia* Brauns 1926 nebst allgemeinen Erörterungen der phylogenetischen Systematik der Megachilidae (Insecta, Hymenoptera, Apoidae). Apidologie 3: 167–186.

Peters, D. S. 1978. Systematik und Zoogeographie der westpalaarktischen arten von *Osmia* Panzer 1806 s. str., *Monosmia* Tkalcu 1974 und *Orientosmia* n. subgen. (Insecta: Hymenoptera: Megachilidae). Senckenb. Biol 58: 287–346.

Prum, R. O. 1990. Phylogenetic analysis of the evolution of display behavior in the Neotropical manakins (Aves: Pipridae). Ethology 84: 202–231.

Proctor, H. C. 1996. Behavioral characters and homoplasy: Perception versus practice, pp. 131–140. *In* M. J. Sanderson and L. Hufford [eds.], Homoplasy. Academic, San Diego, CA.

Queiroz, A., de, and P. H. Wimberger. 1993. The usefulness of behavior for phylogeny estimations: Levels of homoplasy in behavioral and morphological characters. Evolution 47: 46–60.

Raw, A. 1974. Pollen preferences of three *Osmia* species (Hymenoptera). Oikos 25: 54–60.

Roberts, R. R. 1978. The nesting biology, behavior and immature stages of *Lithugus chrysurus,* an adventitious wood-boring bee in New Jersey (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 51: 735–745.

Roig-Alsina, A., and C. D. Michener. 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). Univ. Kans. Sci. Bull. 55: 123–173.

Rozen, J. G. 1970. Biology, immature stages, and phylogenetic relationships of tideline bees, with a description of a new species of *Neofidelia* (Hymenoptera, Apoidea). Am. Mus. Nov. 2427: 1–25.

Rozen, J. G. 1973. Life history and immature stages of the bee *Neofidelia* (Hymenoptera, Fideliidae). Am. Mus. Nov. 2519: 1–14.

Rozen, J. G. 1977. The ethology and systematic relationships of fideline bees, including a description of the mature larva of *Parafidelia* (Hymenoptera, Apoidea). Am. Mus. Nov. 2637: 1–15.

Rozen, J. G. 1991. Evolution of cleptoparasitism in anthophorid bees as revealed by their mode of parasitism and first instars (Hymenoptera: Apoidea). Am. Mus. Nov. 3029: 1–36.

Rust, R. W. 1974. The systematics and biology of the genus *Osmia,* subgenera *Osmia, Chalcosmia,* and *Cephalosmia* (Hymenoptera: Megachilidae). Wasmann J. Biol. 32: 1–93.

Rust, R. W. 1980. Nesting biology of *Hoplitis biscutellae* (Cockerell) (Hymenoptera: Megachilidae). Entomol. News 91: 105–109.

Rust, R. W. 1986. Biology of *Osmia (Osmia) ribifloris* Cockerell (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 59: 89–94.

Rust, R. W., and S. L. Clement. 1972. The biology of *Osmia glauca* and *Osmia nemoris* (Hymenoptera: Megachilidae). J. Kans. Entomol. Soc. 45: 523–528.

Rust, R. W., R. W. Thorp, and P. F. Torchio. 1974. The ecology of *Osmia nigrifrons* with a comparison to other *Acanthosmioides.* J. Nat. Hist. 8: 29–47.

Sinha, R. N. 1958. A subgeneric revision of the genus *Osmia* in the Western Hemisphere (Hymenoptera: Megachilidae). Univ. Kans. Sci. Bull. 34: 211–261.

Stephen, W. P., G. E. Bohart, and P. F. Torchio. 1969. The biology and external morphology of bees. Oregon State University, Corvallis, OR.

Swofford, D. L. 1993. Phylogenetic analysis using parsimony (PAUP) computer program, version 3:1. Illinois Natural History Survey, Champaign, IL.

Taséi, J.-N. 1972. Observations préliminaires sur la biologie d'*Osmia (Chalcosmia) coerulescens* L., (Hymenoptera: Megachilidae), pollinisatrice de la luzerne *(Medicago sativa* L.). Apidologie 3: 149–165.

Taséi, J.-N. 1976. Récolte des pollens et approvisionnement du nid chez *Osmia coerulescens* L., (Hymenoptera: Megachilidae). Apidologie 7: 277–300.

Tinbergen, N. 1959. Behaviour, systematics, and natural selection. Ibis 101: 318–330.

Tkalcu, B. 1975. Revision der europäischen *Osmia (Chalcosmia)*-Arten der *fulviventris*-Gruppe. Vest. Csl. Spol. Zool. 39: 297–317.

Torchio, P. F. 1989. In-nest biologies and development of immature stages of three *Osmia* species (Hymenoptera: Megachilidae). Ann. Entomol. Soc. Am. 82: 599–615.

Vicens, N., J. Bosch, and M. Blas. 1993. Análisis de los nidos de algunas *Osmia* (Hymenoptera, Megachilidae) nidificantes en cavidades preestablecidas. Orsis 8: 41–52.

Wenzel, J. W. 1992. Behavioral homology and phylogeny. Ann. Rev. Ecol. Syst. 23: 361–368.

Wenzel, J. W. 1993. Application of the bigenetic law to behavioral ontogeny: a test using nest architecture in paper wasps. J. Evol. Biol. 6: 229–247.

Westrich, P. 1989. Die Wildbienen Baden-Württembergs. Eugen Ulmer, Stuttgart, Germany.

White, R. J. 1952. A revision of the genus *Osmia,* subgenus *Acanthosmioides* (Hymenoptera: Megachilidae). Univ. Kans. Sci. Bull. 35: 219–307.

Whitman, C. O. 1899. Animal behavior, pp. 285–338. *In* C. O. Whitman [ed.], Biological lectures. Wood's Hole Ginn, Boston.

Yáñez, O. 1997. Biología de *Lithurge (Lithurgopsis) littoralis* Cockerell, 1917 (Apoidea: Megachilidae) en la reserva ecológica del pedregal de San Ángel, D.F. M.S. thesis, Universidad Nacional Autónoma de Mexico.

Yasumatsu, K., and Y. Hirashima. 1950. Revision of the genus *Osmia* of Japan and Korea (Hymenoptera: Megachilidae). Mushi 21: 1–21.

Appendix 1. Loci character matrix (corresponding loci presented at bottom)

Taxa/Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
M. angelarum	1	0	2	2	4	3	3	2	2	4	3	3	4	0	1	2	3	2	4	0	3	0	4	2	2	2	3	3	1	2	1	1	2	3	3	0	2	0
H. adunca	0	0	3	1	1	3	3	3	0	3	1	3	4	0	2	2	3	0	3	0	4	0	4	2	2	1	3	2	1	1	2	1	0	2	4	1	1	0
O. fulviventris	2	0	1	1	1	1	1	1	2	3	1	2	4	2	0	2	2	1	3	0	2	0	1	1	0	1	4	1	1	0	2	2	2	2	1	1	1	0
O. latreillei	2	0	1	1	2	0	1	0	2	3	1	0	4	2	0	2	2	1	2	0	1	0	1	1	0	0	4	1	1	1	2	2	2	2	0	1	1	0
O. lignaria	2	1	1	1	0	0	0	2	2	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	0	2	2	0	0	0	0	1	1	1	0	1	0	1
O. ribifloris	2	0	1	1	1	1	1	2	2	2	1	3	2	0	0	1	1	1	2	0	1	0	3	1	0	2	1	1	0	2	1	2	1	3	1	0	1	1
O. cornuta	2	1	1	1	2	0	1	2	2	2	1	2	1	0	0	1	1	1	1	0	2	0	1	1	0	2	5	1	0	1	1	0	0	0	0	1	0	1
O. rufa	2	1	1	1	2	0	1	1	2	2	1	0	0	0	0	1	1	1	1	0	0	2	1	1	0	2	0	1	2	1	1	2	2	0	1	0	1	1
O. tricornis	2	1	1	1	2	1	1	2	2	2	1	2	3	0	0	1	2	1	2	0	4	0	2	0	0	2	4	1	0	1	1	0	2	1	1	0	1	1
O. cornifrons	2	1	1	1	1	1	1	2	2	2	1	1	2	0	0	1	2	1	1	0	2	0	1	1	0	2	3	1	0	1	1	0	2	1	1	0	1	1
O. taurus	2	1	3	1	2	0	1	0	2	2	1	2	1	0	0	1	2	1	2	0	2	0	2	1	0	2	4	1	0	1	1	0	0	1	1	0	1	1
O. californica	2	0	1	0	2	1	1	2	1	1	1	3	3	0	0	1	2	1	4	2	3	1	2	3	0	1	2	1	2	1	1	2	0	2	3	2	1	0
O. montana	2	0	0	1	2	1	1	2	1	2	1	3	3	0	0	1	2	1	4	2	3	1	2	0	0	1	3	1	2	1	1	2	0	2	3	3	1	0

1 = AK1, 2 = AK2, 3 = CK1, 4 = CK2, 5 = DIA1, 6 = EST1, 7 = ESTF1, 8 = ESTF2, 9 = FBP1, 10 = GAM1, 11 = GAPDH1, 12 = GDA1, 13 = GK1, 14 = GP1, 15 = GP2, 16 = G3P1, 17 = GPI1, 18 = GPI1, 19 = GR1, 20 = G6PDH1, 21 = HA1, 22 = HBDH1, 23 = IDH1, 24 = MDH1, 25 = ME1, 26 = MPI1, 27 = ODH1, 28 = PEP1, 29 = PEP2, 30 = PEP3, 31 = PEP4, 32 = PEP5, 33 = PGD1, 34 = PGM1, 35 = PGM2, 36 = PGM3, 37 = SOD1, 38 = SOD2.

Appendix 2. Nest characters and states

1. ADULT ACTIVITY. 0: Late spring-summer; 1: Spring
2. VOLTINISM. 0: Univoltine; 1: Parsivoltine; 2: Bivoltine
3. OVERWINTERING STAGE. 0: Larva; 1: Adult; 2: Pupa
4. PROTERANDRY. 0: Yes; 1: No
5. NESTING SITE. 0: Burrows; 1: Cavities; 2: Snail shells; 3: Exposed nests
6. NUMBER OF NEST MATERIALS. 0: One; 1: Two
7. MAIN NEST MATERIAL. 0: Wood chips or fibers; 1: Resin; 2: Mud; 3: Masticated plant tissues
8. CELL PARTITIONS. 0: Single; 1: Double
9. CELL WALLS. 0: Not lined; 1: Lined
10. NEST PLUG MATERIAL. 0: Same as cell partition; 1: Additional materials
11. NEST PLUG POSITION. 0: Cavity orifice; 1: Inside cavity
12. VESTIBULE. 0: Absent; 1: Present
13. POLLEN SPECIALIZATION. 0: Oligolectic; 1: Polylectic; 2: Monolectic
14. POLLEN PROVISION—TEXTURE. 0: Dry; 1: Moist, 3: Moist core—dry surface (re-worked provision)
15. POLLEN PROVISION—SHAPE. 0: Cylinder filling most of the cell; 1: Truncated cylinder; 2: Spherical
16. EGG PLACEMENT. 0: In a chamber inside the provision; 1: In a depression on the surface of the provision; 2: On the surface of the provision
17. TIME OF EGG LAYING. 0: Before provision is completed; 1: After provisioning is completed.
18. NUMBER OF EGGS PER PROVISION. 0: One; 1: More than one
19. FECAL PELLETS—PLACEMENT. 0: Scattered about cell; 1: Localized
20. FECAL PELLETS—CONSISTENCY. 0: Pellets retain shape; 1: Pellets smeared
21. FECAL PELLETS—SHAPE I. 0: Flattened; 1: Cylindrical
22. FECAL PELLETS—SHAPE II. 0: Tips truncated; 1: Tips rounded or pointed
23. FECAL PELLETS—SURFACE. 0: Not grooved; 1: Grooved
24. FECAL PELLETS—COLOR. 0: Same as provision; 1: Different from provision
25. COCOON ATTACHMENT. 0: Both cell partitions; 1: Only posterior partition
26. COCOON NIPPLE. 0: Absent; 1: Present, very flat; 2: Present, raised
27. COCOON CAP AROUND NIPPLE. 0: Absent; 1: Present
28. COCOON THICK MIDDLE LAYER. 0: Absent; 1: Present
29. COCOON SILKY OUTER LAYER. 0: Absent; 1: Present

Appendix 3.   **Nest character matrix. Character states are given in Appendix 2**

Species	Characters and states																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Osmia (Osmia)																													
O. lignaria	1	0	1	0	1	0	2	0	0	0	0	1	1	1	1	2	1	0	0	0	0	1	1	1	0	2	0	1	1
O. ribifloris	1	0	1	0	1	0	3	0	0	0	1	1	1	1	1	2	1	0	0	0	0	1	1	0	0	2	0	1	1
O. cornuta	1	0	1	0	1	0	2	0	0	0	0	1	1	1	1	2	1	0	0	0	0	1	1	1	0	2	0	1	1
O. rufa	1	0	1	0	1	0	2	0	0	0	0	1	1	0	1	2	1	0	0	0	0&1	0&1	0&1	1	0	2	0	1	1
O. tricornis	1	0	1	0	1	0	2	0	0	0	0	1	1	1	1	2	1	0	0	0	0	1	1	1	0	2	0	1	1
O cornifrons	1	0	1	0	1	0	2	0	0	0	0	1	1	1	1	2	1	0	0	0	0	1	1	1	0	2	0	1	1
O. taurus	1	0	1	0	1	0	2	0	0	0	0	1	1	0	1	2	1	0	0	0	0	1	1	1	0	2	0	1	1
Osmia (Cephalosmia)																													
O. california	0	1	0&1	0	1	1	2&3	0	0	0	1	1	0	3	0	0	1	0	0	1	1	1	0	0	1	2	1	1	0
O. montana	0	1	0&1	0	1	0	3	0	0	0	1	1	0	3	0	0	1	0	0	1	1	1	0	0	1	2	1	1	0
Osmia (Helicosmia)																													
O. fulviventris	0	1	0&1	0	1	0	3	0	0	0	1	1	0	3	0	1	1	0	0	1	1	0	0	0	1	2	1	1	0
O. latreillei	1	1	0&1	0	1	0	3	0	0	0	1	1	0	3	0	1	1	0	0	1	1	0	0	0	1	2	1	1	0
Outgroups																													
M. angelarum	0	0	0	0	1	1	1	0	1	1	0	1	1	1	1	2	1	0	0	0	1	1	0	0	1	1	0	0	0
H. adunca	0	1	0	0	1	0	2	1	1	0	0	1	0	1	2	2	1	0	0	0	0	1	1	0	1	1	0	0	0